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Research Paper
INT-RP-493

December 1996

Effects of Humidity on Storing Big Sagebrush Seed

Bruce L. Welch

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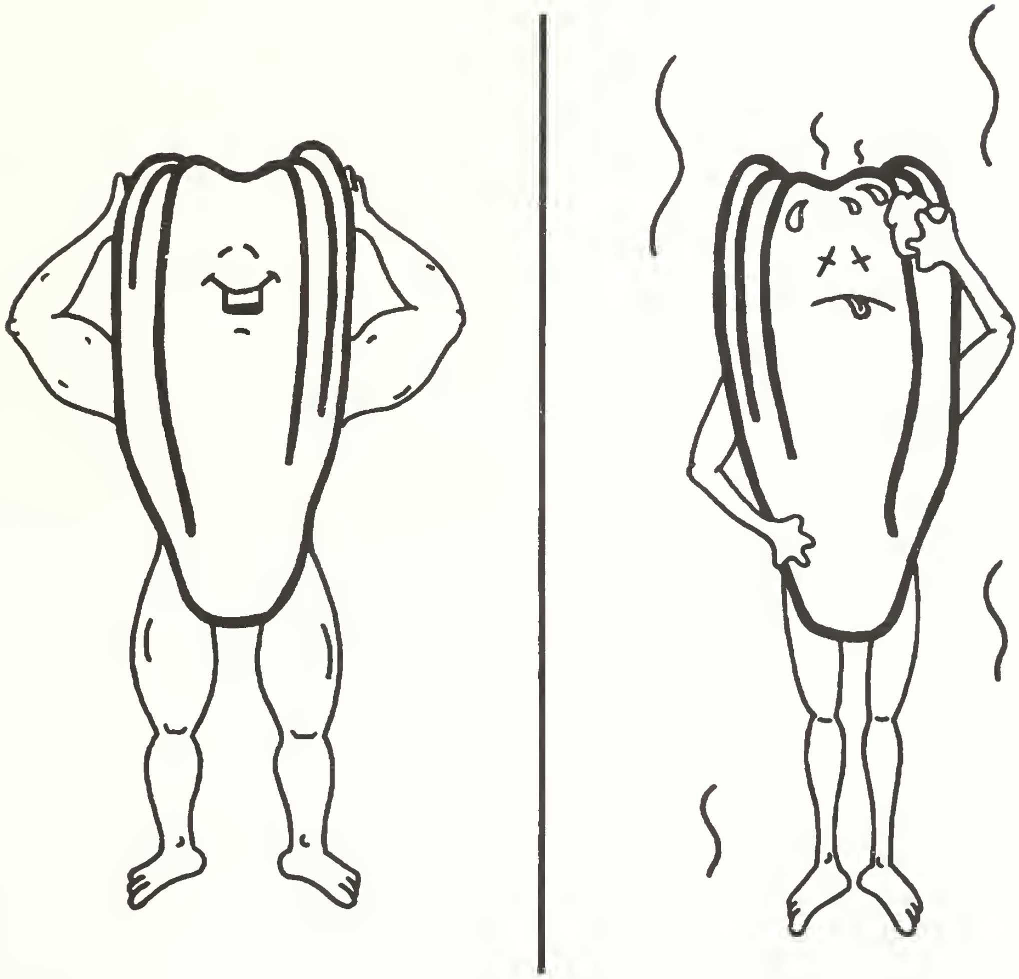

The Author

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Research Summary

Big sagebrush seeds from two sources ('Hobble Creek' mountain big sagebrush—*Artemisia tridentata* ssp. *vaseyana*; Gordon Creek Wyoming big sagebrush—*Artemisia tridentata* ssp. *wyomingensis*) were exposed to different humidities for varying time periods. Results indicated that big sagebrush seed should not be stored in environments with humidities over 40 percent.

A test was performed on 'Hobble Creek' mountain big sagebrush seed to determine heat tolerance. It was found that the seeds could tolerate temperatures up to 60 °C, suggesting that forced-air seed driers should not heat big sagebrush seeds past 60 °C.

Acknowledgments

The author wishes to thank Dr. Dale L. Bartos of the U.S. Department of Agriculture, Forest Service, Intermountain Research Station, Dr. Stanford A. Young of Utah State University, Dr. Darrell J. Weber of Brigham Young University, Dr. Carl L. Wambolt of Montana State University, and Mr. James H. Johansen of the Bureau of Land Management for furnishing peer reviews for this manuscript.

Special thanks goes to Dr. Fred J. Wagstaff (retired U.S. Forest Service) for his encouragement and support.

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Effects of Humidity on Storing Big Sagebrush Seed

Bruce L. Welch

Introduction

Use of big sagebrush (*Artemisia tridentata* Nutt.) seed in revegetation or restoration projects has increased dramatically over the past 10 years (Welch and others 1996, unpublished data). Consequently, a need has developed for information about harvesting, drying, cleaning, and storing big sagebrush seed (Booth and others, in press; Welch 1995). I have received several reports from field personnel indicating that they placed big sagebrush seed of high viability (70 percentage or higher) into storage only to have their viability drop to zero in 6 months or less. Yet, others have reported maintaining big sagebrush seed with a high degree of viability for 3 to 7 years (Stevens and others 1981; Welch and others 1996).

It has long been known that one of several factors that effects longevity of seed in storage is humidity (Barton 1961). High humidity was the likely cause for dramatic reductions in viability in a number of reported cases. This study of the effect of humidity on storing big sagebrush seed came out of these concerns.

The null hypothesis of this study was that the viability of seed in a seed lot stored at different humidities over varying time would not differ. The alternate hypothesis was that the viability of seed in a seed lot stored at different humidities for varying times would differ—that is, decrease—from the start.

Big sagebrush seed is harvested in the late fall or early-to-midwinter months. This is a time of low temperatures and frequent rain or snow. For those who harvest seed by removing inflorescences, drying becomes a difficult task without the use of forced-air driers. The question becomes how much heat can big sagebrush seed tolerate? So another concern of this study is to determine the heat tolerance of big sagebrush seed.

Materials and Methods

Experiment 1

Experiment 1 deals with exposing two lots of big sagebrush seed to different humidities at a constant temperature of 20 °C. The two seed lots consisted of 'Hobble Creek' mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana* [Rydb.] Beetle) and of Gordon Creek Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* Beetle & A. Young) (Welch and others 1986; Welch and others 1992). Both seed lots were collected in November 1994. Inflorescences were removed by use of a handclipper and placed in plastic bags. The inflorescences were then dried by placing the plastic bags in a heated (22 °C) greenhouse. The bags were left open and the inflorescences hand stirred twice each day. After drying, the seed lots were cleaned to 99 percent purity (Welch 1995; Welch and Nelson 1995). At the start of the experiment, 'Hobble Creek' seed had a moisture content of 6.2 percent and a viability of 81 percent, whereas Gordon Creek seed moisture was 8.1 percent and viability 91 percent. Viability determinations are described later in this section.

The containers used to expose the seeds to various humidities were built from three components (fig. 1): a 400 ml wide-mouth jar, 88 mm by 104 mm, with airtight lid; a 50 ml polypropylene beaker, 52 mm by 50 mm; and a 10 ml glass beaker, 27 mm by 32 mm. The 50 ml beakers were inverted and affixed to the internal-bottom of the jars with silicon sealer. Next, the 10 ml beakers in the upright position were affixed with sealer to the bottom of inverted 50 ml beakers. To cure the sealer and drive off all volatile compounds, the containers without lids were placed in an oven at 40 °C for 72 hours.

Seeds were then exposed to the following humidities: 0 percent, open or room, 32, 45, 56, 66, 86, and 100 percent. Zero percent humidity was achieved by placing in the jars and around the 50 ml beakers regenerated anhydrous calcium sulfate; in addition, about 3 g of seeds were placed in the 10 ml beakers. The containers were sealed with airtight lids and placed in a growth chamber set at 20 °C. No lights were operating inside the growth chamber. Open or room humidity was achieved by adding the seeds to the 10 ml beakers and not sealing the containers, while placing them in the growth chamber without any chemicals. Humidity of the growth chamber was monitored by means of a microprocessor-based relative humidity and temperature hygrometer.

Remaining humidities—except 100 percent—were achieved by the use of various saturated salt solutions: 32 percent Calcium Chloride Hexahydrate, 45 percent Potassium Nitrite, 56 percent Calcium Nitrate 4-Hydrate, 66 percent Sodium Nitrite, and 86 percent Potassium Bisulfate (Weast 1976). Saturated salt

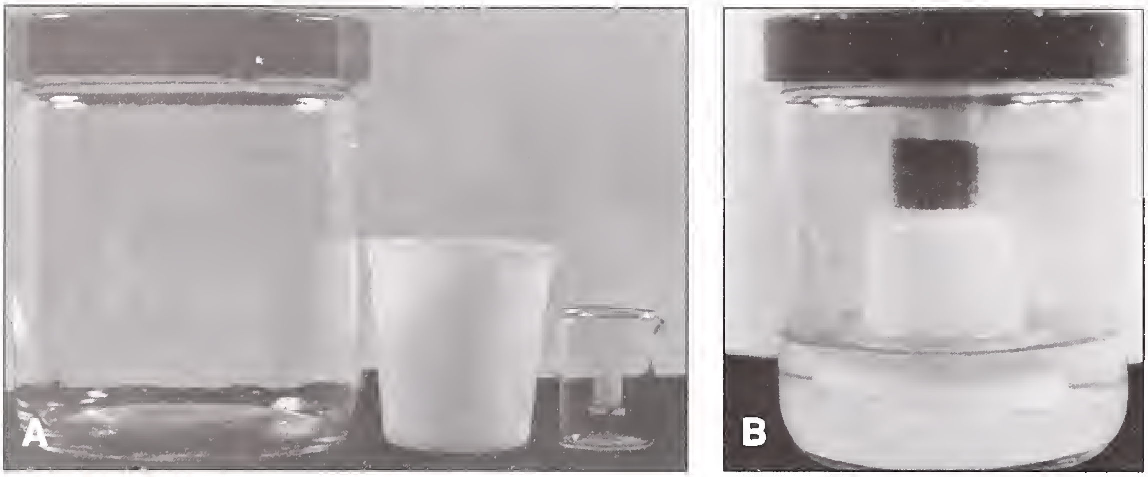

Figure 1—The components used in Experiments 1 and 2. Figure A depicts the three components (400 ml wide mouth jar, 50 ml polypropylene, and 10 ml glass beaker) later assembled into containers used to expose big sagebrush seed to various humidities. Figure B depicts the assembled components with seeds, saturated solution, and airtight lid in place.

solutions to achieve specific humidity levels were prepared by placing 150 ml of deionized water inside the containers. Then, excessive quantities—usually about 100 percent more than what the volume of 150 ml of water can dissolve (Windholz 1983)—of salts were added to the water. Presence of salt crystals is a sign of a saturated salt solution, the desired condition. Humidities of 100 percent were achieved by adding 150 ml of deionized water inside the containers and then closing them with airtight lids.

After the creation of the saturated salt solutions, the containers were sealed with airtight lids and placed in the growth chamber to equilibrate for 2 weeks. Next, about 3 g of seeds were placed inside the 10 ml glass beaker. Before sealing, a sample of seeds out of each container was collected for determining starting seed viability.

Seed viability was determined at 0, 60, 120, 180, 240, 300, and 360 days for each humidity and seed lot. Seed viability was determined by using the tetrazolium staining method (Grabe 1972) as outlined by Meyer and others (1987), except that the seeds were exposed to the buffered tetrazolium solution for 24 hours instead of 6 hours.

The experiment was replicated three times. From each replicate, 300 seeds were used to determine seed viability for a given seed lot-humidity-time. Seed viability means at the start were compared to the seed viability means of given seed lots for the various time and humidities, for example, starting versus 60 days for 32 percent humidity for 'Hobble Creek', starting versus 120 days for 32 percent humidity for Gordon Creek. These two mean comparisons were done by using unpaired, upper-tailed T-tests at the 5 percent probability level (Hintze 1992). The null hypothesis was that the starting viability means of the various humidity-seed lot combinations do not differ from the viability means of the various humidity-seed lot-time combinations. The alternate hypothesis was that the various starting viability means are higher than the various humidity-seed lot-time viability means.

To study the effects of humidity on seed moisture the following test was conducted. Seed moisture was determined for the two seed lots at the start. This was done by placing seed samples into a convection oven set at 100 °C until constant weight was reached and then percent of seed moisture calculated. Next, 1.5 g of seeds were collected at 180 days from each humidity-seed lot combination and seed moisture determined. These two data sets were then used to compare the seed moisture content of the various-seed lot combinations at 180 days. Unpaired, two-tailed T-tests at the 5 percent probability level were used to make the comparisons. The null hypothesis was that seed moisture did not differ from the original seed moisture of the two seed lots and the various humidity-seed lot combinations at 180 days. The alternate hypothesis was that the seed moisture did differ from original seed moisture of the seed lots and the various humidity-seed lot combinations at 180 days.

Experiment 2

Experiment 2 is concerned with exposing big sagebrush seed to different temperatures to determine heat tolerance. A seed lot of 'Hobble Creek' seed stock was selected for this experiment. This lot was cleaned to a purity of 99 percent (Welch 1995). Temperatures that samples of the seed lot were exposed to were: room or control (23 °C), 30, 40, 50, 60, 70, 80, 90, and 100 °C. A forced-air oven was used to generate the various temperatures.

Nine 2 g samples of seed where placed in nine separate glass vials 18 mm by 60 mm and fitted with airtight lids. One vial of seed was assigned at random to each temperature. With the exception of the room or control temperature, the experiment was conducted as follows: the oven was adjusted to the proper temperature and the assigned vial opened and placed in the oven for 48 hours. After the 48 hour exposure, the vials were cooled in a desiccator, fitted with an airtight lid and stored at room temperature until needed for seed viability testing.

For room or control temperature, seed viability was determined at the start of the experiment and then fitted with an air tight lid and stored at room temperature. Seed viability was determined for the various temperatures at 2, 180, and 1,095 days after the exposures. Seed viability was determined as outlined in Experiment 1. Means of room or control temperature were compared to the other temperatures by unpaired, upper-tailed *T*-tests at the 5 percent probability level (Hintze 1992).

Results and Discussion

The results of Experiment 1 are given in tables 1, 2, and 3. Mean growth chamber humidity was 26 percent with a standard deviation of 9.63. These values were

Table 1—Viability of 'Hobble Creek' seed stored at different humidities and for various lengths of time. Data expressed as means and standard deviations of the percent of live seed as determined by tetrazolium staining method. Three replications per humidity-time cell—300 seeds tested per replication. Starting viabilities were compared to the various humidities and time by unpaired, upper-tailed *T*-tests (5 percent probability level).

	At percent humidity							
Days	**0[1]**	**Open[2]**	**32**	**45**	**56**	**66**	**86**	**100**
	Viability[3] (percent)							
0	81 ± 1.7^{a}	81 ± 0.0^{a}	82 ± 1.3^{a}	81 ± 2.1^{a}	81 ± 2.1^{a}	82 ± 2.2^{a}	81 ± 0.8^{a}	81 ± 0.5^{a}
60	80 ± 1.2^{a}	80 ± 1.6^{a}	80 ± 3.1^{a}	63 ± 3.0^{b}	22 ± 2.1^{b}	9 ± 0.8^{b}	0	0
120	79 ± 0.8^{a}	81 ± 1.5^{a}	82 ± 1.7^{a}	57 ± 3.6^{b}	6 ± 1.7^{b}	0	0	0
180	79 ± 3.4^{a}	79 ± 4.1^{a}	79 ± 1.3^{a}	52 ± 2.9^{b}	3 ± 2.6^{b}	0	0	0
240	81 ± 0.8^{a}	85 ± 3.7^{a}	83 ± 1.3^{a}	51 ± 1.9^{b}	2 ± 2.5^{b}	0	0	0
300	85 ± 2.9^{a}	83 ± 1.6^{a}	80 ± 2.2^{a}	39 ± 1.4^{b}	0	0	0	0
360	83 ± 1.5^{a}	84 ± 3.1^{a}	81 ± 2.1^{a}	24 ± 2.6^{b}	0	0	0	0

[1] Zero humidity = seed stored over regenerated anhydrous Calcium Sulfate.

[2] Open = seed stored in open containers in growth chamber (humidity 26 percent).

[3] Values in columns with an ^{a} are not significantly different from the starting (0 days) seed viability. Those with a ^{b} or 0 are significantly different from the starting (0 days) seed viability.

Table 2—Viability of Gordon Creek seed stored at different humidities and for various lengths of time. Data expressed as means and standard deviations of the percent of live seed as determined by tetrazolium staining method. Three replications per humidity-time cell—300 seeds tested per replication. Starting (0 days) viabilities were compared to the various humidities and times by unpaired, upper-tailed *T*-tests (5 percent probability level).

	At percent humidity							
Days	**0[1]**	**Open[2]**	**32**	**45**	**56**	**66**	**86**	**100**
	Viability[3] (percent)							
0	90 ± 0.8^{a}	91 ± 1.4^{a}	92 ± 0.9^{a}	91 ± 1.3^{a}	92 ± 1.6^{a}	91 ± 0.5^{a}	91 ± 2.2^{a}	92 ± 0.0^{a}
60	90 ± 0.5^{a}	91 ± 2.2^{a}	91 ± 2.5^{a}	93 ± 2.5^{a}	64 ± 0.5^{b}	6 ± 1.7^{b}	0	0
120	86 ± 2.5^{a}	91 ± 2.7^{a}	91 ± 3.7^{a}	92 ± 1.6^{a}	57 ± 5.8^{b}	0	0	0
180	86 ± 2.3^{a}	89 ± 1.7^{a}	90 ± 1.7^{a}	90 ± 2.5^{a}	51 ± 1.9^{b}	0	0	0
240	90 ± 0.4^{a}	91 ± 2.6^{a}	92 ± 1.3^{a}	88 ± 2.6^{a}	54 ± 3.4^{b}	0	0	0
300	90 ± 0.0^{a}	89 ± 0.8^{a}	89 ± 2.6^{a}	86 ± 3.7^{a}	24 ± 3.7^{b}	0	0	0
360	92 ± 2.1^{a}	93 ± 2.8^{a}	93 ± 1.9^{a}	89 ± 1.2^{a}	0	0	0	0

[1] Zero humidity = seeds stored over regenerated anhydrous Calcium Sulfate.

[2] Open = seeds stored in open containers in a growth chamber (humidity about 26 percent).

[3] Values in columns with an ^{a} are not significantly different from the starting (0 days) seed viability. Those with a ^{b} or 0 are significantly different from the starting (0 days) seed viability.

Table 3—Percent of moisture of seed exposed to various humidities for 180 days. Seed sources were 'Hobble Creek' and Gordon Creek. Data expressed as means and standard deviations of percent of seed moisture. Seed moisture at the start of the study were compared with the various humidities-source combination by unpaired, two-tailed *T*-tests (5 percent probability).

Humidity	'Hobble Creek'	Gordon Creek
	Moisture[1] *(percent)*	
Start	6.2 ± 0.46^{a}	8.1 ± 0.22^{a}
0[2]	4.5 ± 0.44^{b}	4.4 ± 0.00^{b}
Open[3]	5.1 ± 0.16^{b}	4.6 ± 0.29^{b}
32	5.1 ± 0.08^{b}	4.6 ± 0.22^{b}
45	6.8 ± 0.22^{a}	5.9 ± 0.29^{b}
56	7.7 ± 0.08^{b}	9.4 ± 0.24^{b}
66	9.4 ± 0.14^{b}	9.9 ± 0.31^{b}
86	14.9 ± 2.05^{b}	11.5 ± 0.59^{b}
100	74.6 ± 3.68^{b}	80.0 ± 8.29^{b}

[1] Values in columns with an ^{a} are not significantly different from the starting or control seed moisture. Those with a ^{b} are significantly different from the starting or control seed moisture.

[2] Zero humidity = seeds stored over regenerated anhydrous Calcium Sulfate.

[3] Open = seeds stored in open containers inside a growth chamber (humidity about 26 percent).

based on 156 observations. This means that the open or room humidity was about 26 percent.

For the 'Hobble Creek' seed lot (table 1), significant reduction in seed viability from control or start seed began at 60 days at humidities of 45, 56, 66, 86, and 100 percent. For the Gordon Creek seed lot (table 2), significant reduction in seed viability also began at 60 days, but at humidities of 56 and above. Seed viability of the two seed lots at the start and end of the experiment were not significantly different. Time did not reduce seed viability—test seed were stored in glass vials fitted with airtight lids and stored at room temperature.

The main contrast between the two seed lots is that Gordon Creek seed retained high seed viability at 45 percent humidity throughout the test period, whereas, 'Hobble Creek' seed did not. Gordon Creek seed stored at 56 percent humidity had higher viability than 'Hobble Creek' seed. Why, is unknown? Gordon Creek is a Wyoming big sagebrush, whereas 'Hobble Creek' is a mountain big sagebrush; however, more sources of Wyoming and mountain big sagebrush are needed in order to conclude a subspecies difference.

From the results of this study, it appears that big sagebrush should not be stored in humidities above 32 percent. Although, in a companion study, mountain big sagebrush has been stored for 30 months in a humidity of 40 percent at a temperature of 20 °C without any harmful effect on seed viability (Welch and others 1996). Also, Barton (1961) reported that seed viabilities for a variety of seeds at 20 °C remained higher at 35 percent humidity than at 55 and 76 percent humidities.

Results of the percent of seed moisture after 180 days are given in table 3. For the 'Hobble Creek' seed lot, significant drying occurred at humidities 0 (seed stored over Calcium Sulfate), open (26), and 32 percent. Seed moisture at 45 percent humidity did not differ significantly from the starting seed moisture. Significant hydration occurred at humidities of 56, 66, 86, and 100 percent. These same patterns of drying and hydration were noted for the Gordon Creek seed lot except at 45 percent humidity where Gordon Creek seed was significantly drier than the starting seed moisture. Bewley and Black (1994) reported similar observations for rapeseed, an oil seed. Welch (in preparation) reported that the crude fat content of big sagebrush seed was 28.9 percent of dry matter. This would be enough fat to consider big sagebrush as an oil seed.

An individual reported storing big sagebrush seed with a starting-seed viability of 93 percent. In 6 months, the viability had dropped to zero. Seed samples were sent to the author for viability and moisture testing. Viability was zero with seed moisture at 11 percent. This corresponds to a humidity reading based on the data in this study of 66 to 86 percent. At either humidity, seed viability in this study was zero after 120 days.

Results of Experiment 2 (table 4) shows that no significant reductions in seed viability were detected from 30 to 60 °C for 2, 180, and 1,095 days after treatments. Temperatures above 60 °C reduced seed viability regardless of time. There appears to be a significant reduction of seed viability from 180 to 1,095 days. This observation, however, was not statistically analyzed. These results indicate, forced-air driers should not heat big sagebrush seed past 60 °C. Barton (1961) reported that appropriate drying temperatures for seeds ranged from 43 to 75 °C depending on species.

Conclusions

Apparently big sagebrush seeds should not be stored in environments having greater than 32 to 40 percent humidities. Forced-air driers used to dry seeds should not heat big sagebrush seeds above 60 °C.

Table 4—'Hobble Creek' seed viability 2 days, 180 days, and 1,095 days after being exposed to various temperatures for 48 hours. Data expressed as percent of live seeds as determined by the tetrazolium staining method. Three replications per temperature setting—300 seeds were tested per replication. Seed viability of seed kept at room temperature—control—were compared with seed viability of the various temperatures cells by unpaired, upper-tailed *T*-tests (5 percent probability level).

	Days after exposure		
Temperatures	**2**	**180**	**1,095**
	- - - - - - - - - *Viability*[1] *(percent)* - - - - - - - - -		
Control	91 ± 2.08^a	91 ± 0.00^a	73 ± 3.06^a
30°	89 ± 2.08^a	90 ± 2.00^a	74 ± 0.58^a
40°	89 ± 3.61^a	89 ± 2.85^a	77 ± 1.00^a
50°	86 ± 2.65^a	84 ± 2.82^a	75 ± 0.00^a
60°	88 ± 1.02^a	86 ± 2.94^a	76 ± 0.58^a
70°	79 ± 7.94^b	81 ± 1.53^b	65 ± 3.61^b
80°	75 ± 4.04^b	70 ± 2.08^b	62 ± 2.65^b
90°	25 ± 3.79^b	0	0
100°	0	0	0

[1] Values in columns with an ^{a} are not significantly different from the room temperature (control) seed viabilities. Those with a ^{b} or 0 are significantly different from the room temperature (control) seed viabilities.

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Data support the conclusion that big sagebrush seed should not be stored in environments having humidities above 32 to 40 percent. Some humidities may even dry seed to a greater degree than when first placed in storage. Forced-air seed driers should not heat big sagebrush seed over 60 °C.

Keywords: *Artemisia tridentata*, 'Hobble Creek', mountain big sagebrush, Gordon Creek, Wyoming big sagebrush, *Artemisia tridentata* ssp. *vaseyana*, *Artemisia tridentata* ssp. *wyomingensis*

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